Evaluating the effect of the dopamine receptor D2 on signaling of the neurotensin receptor 1

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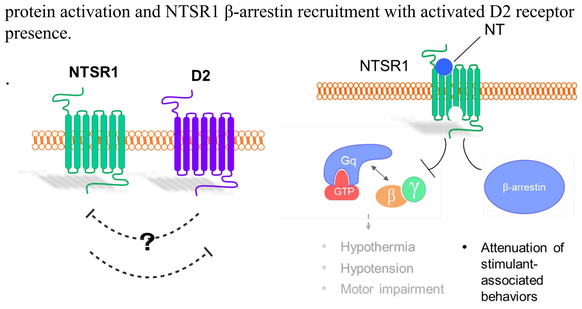
Introduction

Meth is one of the most commonly misused drugs in the world with no FDA-approved therapeutics.According to the 2023 National Survey on Drug Use and Health (NSDUH), more than 16 million people aged 12 or older used methamphetamine in their lifetime[1]. There is an urgent need to find possible treatments for Meth Use Disorder (MUD). Meth increases synaptic dopamine (DA) and induces dysfunction of central dopaminergic neurotransmission.[2] However, direct blockade of dopamine signaling is not a viable treatment strategy as dopamine plays a diverse role in the central nervous system. Dopamine homeostasis may be accomplished by targeting the neurotensin receptor 1 (NTSR1)[2]. NTSR1 is commonly co-expressed with the dopamine receptor 2 (D2) in dopamine-rich brain regions and has been shown to attenuate the behavioral effects of psychostimulants (e.g., meth and cocaine)[2]. Understanding the relationship between NTSR1 and D2 could help find promising treatments for MUD[3]. Both NTSR1 and D2 are cell surface G protein-coupled receptors (GPCRs). Balanced NTSR1 agonists have been shown to attenuate the actions of dopamine via a functional and putative physical NTSR1-D2 receptor-receptor interaction. It has been demonstrated that NTSR1 ligands have an antagonist effect on D2 receptors, specifically in reducing D2 G protein signaling[4]. However, the effect that D2 receptor activation has on NTSR1 signaling and trafficking remains unknown. This research will provide a more comprehensive understanding of the relationship between these two receptors. My central hypothesis is that D2 and NTSR1 have a mutually antagonistic relationship and that D2 attenuates NTSR1 signaling.We will determine the effect of D2 receptor activation on NTSR1 signaling and trafficking through examining NTSR1 G protein activation and NTSR1 β-arrestin recruitment with activated D2 receptor presence.



Objectives

- **Assess the effect of D2 activation on NTSR1 G protein signaling.**
- **Assess the effect of D2 activation on NTSR1 β-arrestin recruitment.**
- **Determine the cell types in the mouse brain that express both NTSR1 and D2.**
- **Determine the cell types in the mouse brain that express both NTSR1 and D1.**

Methods

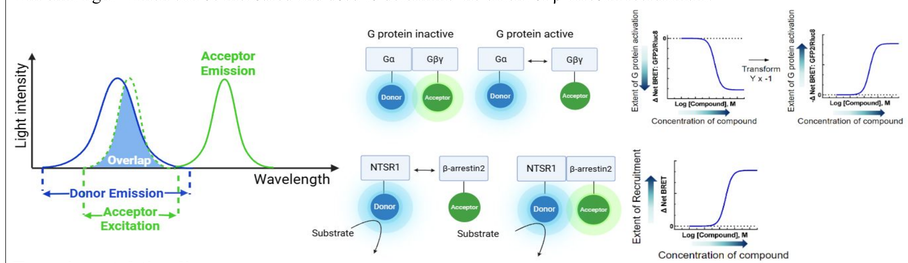
NTSR1 DRD1 and DRD2 co-expression cells analysis : We used the Allen Brain Istitute's ABC Atlas Brain Knowledge Platform to analyze co-expression cells by different threshold and cell classes, and calculated using following formula:

1. Percentage of Co-expressing Cells in GPCRs cells= Total co-expressing Cells in the threshold/total number of GPCRs cells in the selected region × 100%.
2. Percentage of co-expressing GPCRs cells in specific cell class= (co-expressing GPCRs cells in specific cell class/ Total Cells in the Cell class) × 100%.

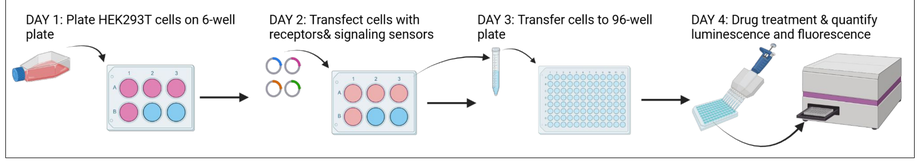
Methods

Assessment of NTSR1 G protein activation using BRET2: NTSR1 G protein activation in HEK293T cells is measured by Bioluminescence resonance energy transfer(BRET), a proximity-based protein-protein interaction assay. By using this assay to monitor the association of a BRET donor-tagged Gα and a BRET acceptor-tagged Gβγ, as the receptor activated, the renilla luciferase-tagged Gα and the mVenus-tagged Gβγ will separate[8], resulting in loss of fluorescence which can be used to determine the extent of G protein activation.

Assessment of NTSR1 β-arrestin recruitment using BRET1 : NTSR1 β-arrestin recruitment in HEK293T cells is measured by BRET assay. By monitoring the NT-stimulated association of NTSR1-Rluc8 and β-arrestin-venus, we can measure the association of a renilla luciferase tagged to a G protein-coupled receptor (GPCR) with a venus tagged to β-arrestin. Upon activation, the venus-tagged G alpha protein will come in close proximity with the receptor. Once the renilla luciferase (BRET donor) and the venus (BRET acceptor) are within 50 nanometers of each other, these molecules will emit light[8] which can be measured and used to determine the extent of β-arrestin recruitment.

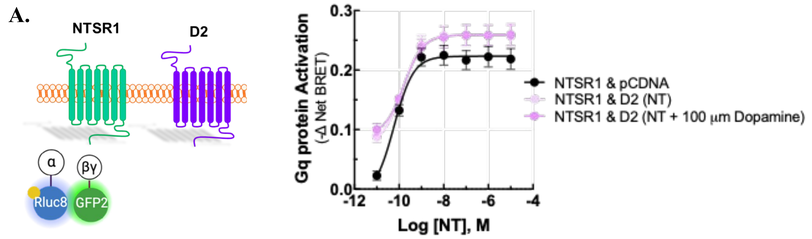


Experimental timeline:



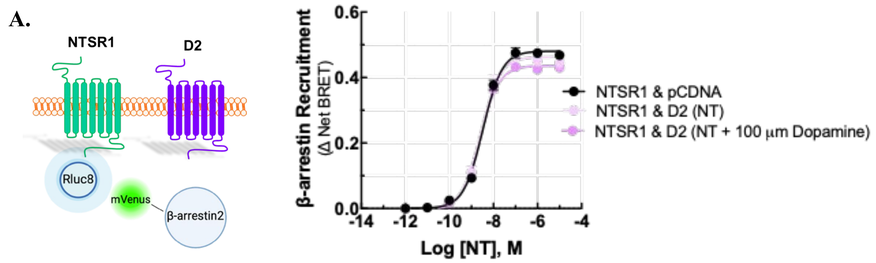
Results

I. Dopamine-induced D2 activation increases NTSR1 Gq protein activation



NT induced $G_{\alpha}q$ activation by the NTSR1 is enhanced by Dopamine induced D2 receptor activation. (A) Cartoon showing assay components. (B) NTSR1 co-expressed with D2 enhances Gαq activation by the NTSR1 regardless of different concentration of dopamine treatment.

II. Dopamine-induced D2 receptor activation has no effect on neurotensin-induced β-arrestin recruitment to the NTSR1



β-arrestin recruitment to the NTSR1 is unaffected by Dopamine induced D2 receptor activation. (A) Cartoon showing assay components.. (B) D2 activated by dopamine shows similar results to inactive D2.

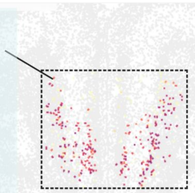
Results

III. NTSR1 D1 and D2 co-expression analysis in prelimbic & infralimbic regions using ABC Atlas Brain knowledge platform [5]

A

NTSR1 & DRD2 co-expression

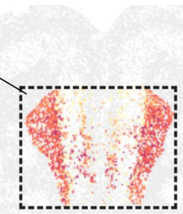
In the prelimbic & infralimbic regions, approximately 8.8% of the NTSr1 cells (above the Log2(CPM+1) > 1.10 threshold) express DRD2(DRD2 threshold range: 0.966 <Log(CPM+1)< 9.656), and approximately 69% of the DRD2 cells (DRD2 threshold range: 0.966 <Log(CPM+1)< 9.656) express NTSr1, suggesting that the co-expressing cells are commonly found in the DRD2 expressed cells/ majority of DRD2 expressed cells can expressed NTSr1, however only a small proportion of NTSr1 cells can expressed DRD2.



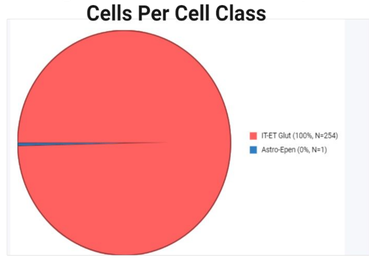
B

NTSR1 & DRD1 co-expression

In the prelimbic & infralimbic regions, approximately 75.5% of the NTSR1 cells (above the Log2(CPM+1) > 1.10 threshold) expressed DRD1 and approximately 73.2% of the DRD1(DRD1 threshold range: 0.900 <Log(CPM+1)< 9.000) expressed NTSR1(above the Log2(CPM+1) > 1.10 threshold), suggesting that co-expressing cells can be commonly found in both NTSR1 and DRD1 alone expressed cells/ majority of DRD1 expressed cells can expressed NTSr1, and a similar proportion of NTSr1 cells can also expressed DRD1.

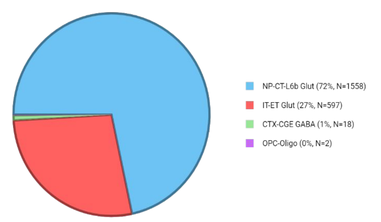


Percentage of NTSR1&DRD2 co-expressed Cells Per Cell Class



Most NTSR1& DRD2 co-expression cells (~99.6%) in the prelimbic& infralimbic regions are likely glutamatergic. The majority of NTSR1&DRD2 cells in the prelimbic & infralimbic regions (99.6%) belong to the IT-ET Glut class.

Percentage of NTSR1&DRD1 co-expressed Cells Per Cell Class



Most NTSR1& DRD1 co-expression cells (~99.1%) are likely glutamatergic,The majority of NTSR1&DRD1 cells (71.63%) belong to the NP-CT-L6b Glut class, and 27.45% of NTSR1&DRD1 co-expressed cells belong to the IT-ET glut class

In prelimbic and infralimbic areas, NTSR1 is expressed by neurons that express both the D2 receptor (DRD2 gene) and the D1 receptor (DRD1 gene). . (A) The majority of NTSR1 & DRD2 coexpression was identified in IT-ET GluT, excitatory glutamatergic subtype (Layer 2/3, Layer 5 intro-telencephalic-Extra-telencephalic), whereas the majority of NTSR1 & DRD1 coexpression was identified in the NP-CT-L6b Glut, excitatory glutamatergic subtype (Near-Projecting Corticothalamic, Layer 6b). (B) NTSR1 and DRD1 are more widely co-expressed than NTSR1 and DRD2.

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Summary

I. Dopamine-induced D2 activation enhances G protein activation by NTSR1.
II. Dopamine-induced D2 receptor activation has no effect on β-arrestin recruitment to the NTSR1.
III. The number of cells co-expressing NTSR1 & D2 in the prelimbic and infralimbic regions is lower than that co-expressing NTSR1 & D1 and belong to a different cell classes. Future studies should expand the investigation of NTSR1-dopamine receptor interactions to include the D1.

References

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Acknowledgements

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